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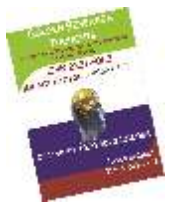

SYNERGISTIC CURATIVE ROLE OF CAFFEINE (1,3,7-TRIMETHYLEXANTHINE) AND L-ASCORBIC ACID ON ARSENIC INDUCED BIOACCUMULATION IN FRESHWATER BIVALVE *LAMELLIDENS CORRIANUS* (LEA)

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ABSTRACT

The present communication deals with the individual and synergistic effectiveness of caffeine (1,3,7-trimethylexanthine) with L-ascorbic acid in arsenic induced toxicity in an experimental model, the freshwater bivalves, *Lamellidens corrianus*. The effect on bivalves was studied under nine groups. Group A bivalves were maintained as control, group B bivalves were exposed to chronic dose of sodium arsenate (0.18ppm) for 20 days, group C bivalves were exposed to respective chronic concentration of sodium arsenate along with of caffeine (1mg/l), Group D bivalves were exposed to sodium arsenate with L-ascorbic acid (25mg/l), Group E bivalves were exposed to respective chronic concentration of sodium arsenate with caffeine + ascorbic acid. Arsenic bioaccumulation was estimated in whole soft body from each group after 10 & 20 days. Bivalves from group B were divided for recovery into four groups F, G, H & I after 20 days exposure to arsenic. F group bivalves were allowed to cure in normal water, G group bivalves were exposed to caffeine (1mg/l), H group bivalves were exposed to ascorbic acid (25mg/l) for recovery while I group bivalves were exposed to caffeine with ascorbic acid. From each recovery groups, after 5 and 10 days some bivalves were removed & arsenic content in whole soft body of bivalves were estimated, arsenic bioaccumulation was significantly higher on exposure to arsenic while the decrease in presence of caffeine + ascorbic acid was more when exposed simultaneously than when exposed individually. During recovery lead content recovered and the rate of recovery was faster in caffeine+ ascorbic acid exposed bivalves as compare to those recovered individually and in normal water. The probable role of the caffeine (1, 3, 7,- trimethylxanthine) and L-

ascorbic acid is discussed in the paper.

KEY WORDS: synergistic, caffeine, ascorbic acid, arsenic, bioaccumulation, *Lamellidens corrianus*

INTRODUCTION:

Bioconcentration is the specific bioaccumulation process by which the concentration of a chemical in an organism becomes higher than its concentration in the air or water around the organism. Although the process is the same for both natural and manmade chemicals, the term bio-concentration usually refers to chemicals foreign to the organism. The biological accumulation of metals by the aquatic organisms pose a serious problem to human population, thus bioaccumulation studies are important in the estimation of potential environmental harm. Toxicants like heavy metal ions and most of pesticides are non degradable, resulting in to bioaccumulation within the ecosystem and its biotic components. Metals are released in to water through anthropogenic activities such as industry and agriculture (Saygider, 2000).

Widespread arsenic poisoning from drinking water is present in West Bengal, India (UNCF, 1998), and neighboring Bangladesh (Khan *et.al,* 1997). Millions of people are exposed to arsenic and more contaminated areas along the Ganges River delta were recently identified (Chakraborti *et.al.,* 2003). Naturally occurring arsenic that contaminates drinking water is the source of this ongoing global public health problem.

According to WHO (1996) arsenic in the drinking water above 0.05 mg/l has health hazards. Raveera et.al., (2003) reported arsenic contents in shells of bivalves, *Unio, Anodonta and Dreissena* body respectively. Arsenic and its oxides are general protoplasmic poisons. Arsenic compounds may cause nausea, vomiting, diarrhea and abdominal pain, sometimes coma and death. Chronic poisoning occurs due to inhalation of fumes or dust containing arsenic compounds. This can result in burning pain of limbs, damage of liver and jaundice and ulceration of skin.

The biological accumulation of metals by the aquatic organisms pose a serious problem to human population, thus bioaccumulation studies are important in the estimation of potential environmental harm. The biological accumulation of metals by the aquatic organisms pose a serious problem to human population, thus bioaccumulation studies are important in the estimation of potential environmental harm. The biological accumulation of metals by the aquatic organisms pose a serious problem to human population, thus bioaccumulation studies are important in the estimation of potential environmental harm. The biological accumulation of metals by the aquatic organisms pose a serious problem to human population, thus bioaccumulation studies are important in the estimation of potential environmental harm.

The term chelation comes from the Greek meaning crab or lobster claw, suggested by the way in which the metal is gripped in at least two places by the organic groups. Chelating agents are used to produce stable compounds with relatively low toxicity and also to enhance the excretion of metals. Heavy metals can also exist covalently bound to organic molecules. In the case of arsenic, this substantially reduces its toxicity.

Ascorbic acid is a diffusible biological reductant when present in appropriate concentration. L-ascorbic acid is a strong antioxidant and may extent its protective role by chelating the metal or by reducing the free radicals and removing them from system. Caffeine is well known nervous system stimulant but beside it, it is now proved that it has antioxidant activity. Caffeine has the capacity to bind with heavy metals as caffeine contains uncharged or negatively charged molecules and that of heavy

metals are positively charged. This property of caffeine indicates that caffeine can play protective role against heavy metals toxicity in living organism.

In the present study, it is proposed to study the protective and curative role of caffeine and ascorbic acid against heavy metal bioaccumulation in an experimental bivalve, *Lamellidens corrianus.*

Materials and Methods:

The freshwater bivalves, *Lamellidens corrianus* were collected from the Nathsagar dam at Paithan, Aurangabad (M.S.). Bivalves were acclimatized in the laboratory condition at room temperature for 2-3 days. The healthy and active acclimatized bivalves of approximately same size were selected for experiment. The effect on bivalve was studied under nine groups. Group A bivalves were maintained as control, B group bivalves were exposed to chronic dose (LC50/10) of sodium arsenate (0.18ppm) for 20 days. Group C bivalves were exposed to respective chronic concentration of sodium arsenate along with caffeine (1mg/l), Group D bivalves were exposed to respective chronic concentration of sodium arsenate along with L-ascorbic acid (25 mg/L.). Group E bivalves were exposed ---to respective chronic concentration of sodium arsenate along with caffeine + ascorbic acid. Bivalves from group B were divided for recovery into four groups F, G, H and I after 20 day exposure to lead. F group bivalves were allowed to cure in normal water, G group bivalves were exposed to caffeine (1mg/l), H group bivalves were exposed to ascorbic acid (25 mg/L) for recovery while I group bivalves were exposed to caffeine (1mg/l) with ascorbic acid (25mg/l).

During treatment whole soft body form each group bivalves were removed after 10 and 20 days. Similarly during recovery after 5 days and 10 days whole soft body were taken from recovery groups. Whole soft body from all experimental and recovery group were dried at 80 0C in an oven until constant weight was obtained. The dried powders of control, experimental and recovery group animals were used for estimations of their respective heavy metal contents.

500 mg of whole soft body tissue powder of control and experimental bivalves was digested in 10 ml. of acid mixture (HNO3: Perchloric acid) in (4:1) ratio on hot plate until dryness. The digested mixture was cooled and diluted to 50 ml by double distilled water in volumetric flask and was filtered by (Whatman grade 541) filter paper. From each sample, arsenic heavy metal was estimated by Atomic Absorption Spectrophotometer (Chemito.).

The concentration of arsenic accumulated in whole soft body of the bivalves from treated and recovery groups were estimated and are given in the table.

Results and Discussion:

From the result of present work it is concluded that Caffeine and Ascorbic acid acts as detoxifying agents as they reduces the metallic ions rapidly by excreting them as a result ascorbic acid levels of tissue is decreases. Synergistically Caffeine and ascorbic acid recovery acts more effectively for detoxification of heavy metals than that of their individual recovery.

Kelepertsis and Alexakis (2004) studied the coastal bottom sea sediments and observed variation of heavy metals, natural variation and subsequent redistribution of the wastes by erosion, transport and deposition. Various species of bivalves have been studied as biological indicator organisms to monitor marine environmental pollution by heavy metal and chemicals due to their own properties of inhabitation (Al-mafda *et.al.,* 1998; Pempkowiak *et.al.*, 1999 and Kuvan *et.al.,* 2002). Ezemonye *et. al.,* (2006) studied the levels and bioaccumulation of copper, zinc and iron in various body organs, viz: viscera and shell of freshwater snail, Pila ovate.

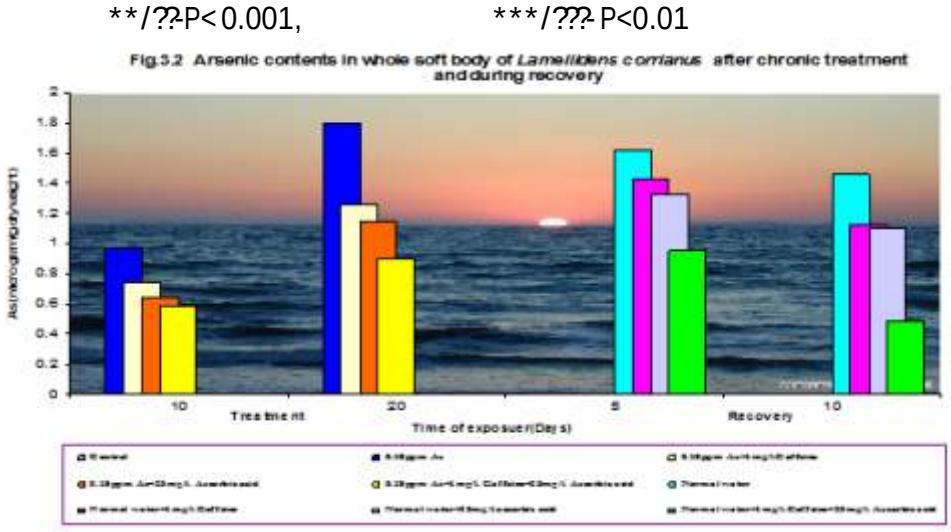
Liao and Ling (2003) carried out probabilistic risk analysis methods to quantify arsenic (As) bioaccumulation in cultured fish tilapia, *Orechromis mossambicus* and large-scale mullet *Liza macrolepis* at blackfoot disease (BFD) area in Taiwan and to assess the range of exposures for the people who eat the contaminated fish. The models implemented include a probabilistic bioaccumulation model to account for As accumulation in fish and a human health exposure and risk model that accounts for hazard quotient and lifetime risk for humans consuming contaminated fish.

Table : Arsenic content (µg/g dry weight) in whole soft body of L. corrianus after chronic exposure to sodium arsenate without and with caffeine, ascorbic acid, with caffeine + ascorbic acid and during recovery

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		0.002 ±0.00	0.002 ±0.00		
0.18 ppm Sodium arsenate		0.960 ±0.003***	1.803 ±0.12***		
0.18 ppm Sodium arsenate +1 mg/l Caffeine		0.748 ±0.06*** (-22.08)	1.260 ±0.09** (-30.11)		
0.18 ppm Sodium arsenate + 25 mg/l Ascorbic acid		0.646 ±0.056*** (-32.70)	1.154 ±0.075** (-35.99)		
0.18 ppm Sodium arsenate + 1 mg/l Caffeine + 25 mg/l Ascorbic acid		0.583 ±0.041*** (-39.27)	0.897 ±0.071*** (-50.24)		
After 20 days exposure to Sodium arsenate (0.18 ppm)	Normal Water			1.619 ±0.11NS [-10.20]	1.455 ±0.125?? [-19.30]
	Normal Water +1mg/l Caffeine			1.432 ±0.135? [-20.57]	1.125 ±0.098?? [-37.60]
	Normal Water + 25 mg/l Ascorbic acid			1.325 ±0.87?? [-26.51]	1.102 ±0.78??? [-38.87]
	Normal Water + 1mg/l Caffeine + 25 mg/l Ascorbic acid			0.952 ±0.684??? [-47.19]	0.489 ±0.235??? [-72.87]

+ indicates standard deviation
Values in ( ) brackets indicate percent changes over respective arsenic treated
Values in [ ] brackets indicates percent change over 20 days arsenic treated
NS - Non significant, *-compared with respective arsenic treated, ?- compared with arsenic treated of 20 days
*/?- P< 0.005, **/??-P< 0.001, ***/???- P<0.01

Fig.3.2 Arsenic contents in whole soft body of *Lamellidens corrianus* after chronic treatment and during recovery



Mahajan, (2005) studied the bioaccumulation of Hg, As and Pb in freshwater gastropod, *Bellamya (Viviparous)* bengalensis and observed that metal accumulation is less when exposed with caffeine in presence of respective concentration of only heavy metals. Gupta *et.al.*, (2002) reported that lead nitrate induced a fall in TEC concentration and Hb% of H. fossils fish. Khanduja *et.al.,*(1999) reported that caffeine and quercetin have anticarcinogenic effect against N-nitrosamine, polycyclic aromatic hydrocarbons and other carcinogens responsible for induction of tumors in animals. Loety et al., (2001) observed that caffeine binds divalent cations of calcium which indicates that caffeine can bind other reactive divalent cations of heavy metals. Shahidi *et. al.,* (1992) Studied that under certain condition phenolic antioxidants can initiate an auto-oxidative process and acts like prooxidants. Caffeine being water soluble and common cheaper beverage, it will be cheapest preventive and curative medicine.

Ascorbic acid serves an immense important role in distribution and excretion of trace minerals and toxic metals (Lewis, 1976). Ascorbic acid acts as detoxifing agent by forming poorly ionized but soluble compounds with Pb (Pillemer *et.al,* 1940). Houston and Johnson (2000) and Naresh *et.al.,*(2003) observed relationship between lead and ascorbic acid level of human blood. Mahajan (2007) in freshwater bivalve L. marginalis found that heavy metal accumulation of As, Cd and Pb was increased with increase in exposure period and it was less when exposed with ascorbic acid in presence of respective concentration of heavy metals as compared to those exposed to only heavy metal. Ascorbic acid is a strong antioxidant and may extends its protective effects by chelating the metals or by precipitating free radicals and removing them from the system

The result profiles indicate the protective and curative role of caffeine and ascorbic acid, individually and in synergistic way on heavy metal bioaccumulation in whole soft body of experimental bivalve, Lamellidens corrianus.

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